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Triggering of Innate Immune Responses by Schistosome Egg Glycolipids and Their Carbohydrate Epitope GalNAc β 1-4(Fuc α 1-2Fuc α 1-3)GlcNAc

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To investigate the interactions of glycoconjugates with the innate immune system, peripheral blood mononuclear cells were stimulated with glycolipids derived from *Schistosoma mansoni* eggs and worms and with biochemically synthesized neoglycoconjugates. Egg glycolipids stimulated the production of interleukin (IL)-10, IL-6, and tumor necrosis factor- α in monocytes, whereas worm glycolipids failed to do so. When monoclonal antibodies that specifically recognize defined carbohydrate epitopes were used, the binding of a GalNAc β 1-4(Fuc α 1-2Fuc α 1-3)GlcNAc (LDN-DF) reactive antibody was pronounced on egg glycolipids but was absent on worm glycolipids. The binding of antibodies that recognize Gal β 1-4(Fuc α 1-3)GlcNAc (LewisX), GalNAc β 1-4GlcNAc (LDN), and GalNAc β 1-4(Fuc α 1-3)GlcNAc (LDN-F) was comparable for both preparations. Cytokine production in response to neoglycoconjugates containing enzymatically synthesized glycans also was measured. The LDN-DF neoglycoconjugate was the most potent cytokine inducer, which indicates that this difucosylated glycan can act at the host-parasite interface and can trigger innate immune responses.

The development of an immune response to a pathogen requires that the cells of the immune system recognize the invader, a process that involves signature molecules of the pathogen, on the one hand, and specific receptors of the host cells, on the other hand. Considerable progress is being made in the area of immune pattern recognition, and the molecular patterns for viruses and bacteria have been characterized and shown to include peptidoglycans and lipopeptides [1–3]. Helminths, which are complex multicellular pathogens, can be present in different developmental stages within a human host, and each stage may carry glycoconjugates that bear novel combinations of glycans [4–6]. An attractive proposition is that such carbohydrate motifs can interact with the immune system and transmit information on the presence of different life cycle stages of helminths, setting the scene for the development of a pro- or an anti-inflammatory im-

mune response. Schistosomes are trematodes that cause schistosomiasis, a chronic blood-vascular disease that has been studied intensively at the T and B cell level. Studies of humans, as well as of animal models of schistosomiasis, have shown that there are dynamic changes in pro- and anti-inflammatory responses that are crucial to the development or containment of immunopathologic processes [7–10]. It is clear that immune responses directed to parasite eggs trapped in host tissues lead to granuloma formation and, in a proportion of the population, these responses can progress to chronic disease. Thus, the immunological events that occur immediately after a first encounter with different parasite life cycle stages and their signature molecules may determine the outcome of an infection, which emphasizes the importance of identifying such parasite molecules and their interactions with the immune system.

Schistosomes contain a rich array of glycoproteins and glycolipids that have been the subject of several recent structural studies (reviewed in [11]). The best studied structure of helminth glycoproteins is Gal β 1-4(Fuc α 1-3)GlcNAc, the LewisX antigen [12, 13], which has been shown to induce interleukin (IL)-10 production by peripheral blood mononuclear cells (PBMC) of schistosome-infected individuals [14] and, in murine models, to skew immune responses toward Th2 responses [15]. In addition, many parasite-derived glycoconjugates contain the GalNAc β 1-4GlcNAc (LacdiNAc [LDN]) motif [12] and GalNAc β 1-4(Fuc α 1-3)GlcNAc (fucosylated LDN [LDN-F]) derivatives [16]. These structures have been shown to be important antibody-binding targets [17–19]; however, the potential of these structures to stimulate cellular responses has not been investigated. Carbohydrate moieties in schistosomes do not occur on proteins

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Informed consent was obtained from each healthy control subject before blood withdrawal. Human experimentation guidelines of the scientific and ethics committees of all institutions were followed in the conduct of this research.

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only but also can be lipid bound [20]. We showed elsewhere that schistosome glycolipids interact with the immune system at the antibody level [21]. To determine whether these glycolipids and the associated glycans are capable of stimulating cells involved in the innate immune response to schistosomes, we measured the production of early cytokines involved in the pro- and anti-inflammatory network by stimulating PBMC of nonexposed individuals with schistosome glycolipids extracted from eggs and adult worms. By using monoclonal antibodies (MAbs) that specifically recognize defined carbohydrate epitopes, we demonstrated the presence of various glycans on these parasite glycolipids. To investigate the immunomodulatory properties of these glycan structures, we measured cytokine production in response to well-defined neoglycoconjugates containing enzymatically synthesized carbohydrate moieties.

Materials and Methods

Preparation of glycolipids. *Schistosoma mansoni* adult worms were collected by perfusion of golden hamsters (Harlan) 45–48 days after infection. *S. mansoni* eggs were isolated from the livers of infected hamsters after treatment of the liver homogenate with trypsin [22]. Glycolipid preparations were made from *S. mansoni* eggs (3.7×10^6 eggs) and adult worms, as described elsewhere [21]. In brief, a total lipid extract was made according to the method described by Bligh and Dyer [23]. Lipids were separated into different classes by use of triethylaminoethyl cellulose column chromatography (Serva), as described by Rouser et al. [24]. The following fractions contained the specified lipids (the dry weight of egg lipid fractions is indicated): (1) cholesterol, glycerides, and other neutral lipids, 19.4 mg; (2) cerebrosides, glycerol diglycerides, phosphatidyl choline, and sphingomyelin, 14.8 mg; (3) ceramidepolyhexosides, 9.1 mg; (4) inorganic substances, 1.8 mg; (5) phosphatidyl ethanolamine and free fatty acids, 2.4 mg; (6) phosphatidyl serine, 2.2 mg; (7) none (washing step), and (8) phosphatidic acid, cardiolipin, phosphatidyl glycerol, phosphatidyl inositol, and other acidic lipids, 36 mg. The presence of carbohydrates in the ceramidepolyhexoside-containing fraction was confirmed by orcinol staining of this fraction on high-performance thin-layer chromatography plates (Merck).

Biochemical synthesis of oligosaccharides and glycoconjugates. The spacer-linked LewisX, LDN, LDN-F, and GalNAc β 1-4(Fuc α 1-2Fuc α 1-3)GlcNAc (difucosylated LDN [LDN-DF]) oligosaccharides (figure 1) were prepared enzymatically from the synthetic acceptor structure GlcNAc β 1-O-(CH₂)₈COOCH₃ (GlcNAc-R), as described elsewhere [4, 25]. In brief, GlcNAc-R was incubated with UDP-GalNAc and partially purified UDP-GalNAc:GlcNAc β 1 \rightarrow 4-*N*-acetylgalactosaminyltransferase from *Lymnea stagnalis* albumen glands. The reaction product, LDN-R, was purified from the incubation mixture, and a portion was fucosylated by incubation with GDP-Fuc and GDP-Fuc:[Gal β 1 \rightarrow 4]GlcNAc β α 1 \rightarrow 3-fucosyltransferase from human milk, to yield the LDN-F-R trisaccharide. Purified LDN-F-R was further converted to LDN-DF-R, using GDP-Fuc and GDP-Fuc:Fuc α α 1 \rightarrow 2-fucosyltransferase from *Trichobilharzia ocellata* cercariae. In a similar reaction scheme, GlcNAc-R was incubated with UDP-Gal and UDP-Gal:GlcNAc β 1 \rightarrow 4-galactosyltransferase

from bovine milk to yield Gal β 1 \rightarrow 4GlcNAc-R, which subsequently was converted to LewisX-R by use of GDP-Fuc and GDP-Fuc:[Gal β 1 \rightarrow 4]GlcNAc β α 1 \rightarrow 3-fucosyltransferase from human milk. The products of the incubations were isolated and purified by sequential Sep-Pac C-18 (Waters) and Bio-Gel P-4 (Biorad) chromatography, and the chemical structures of the purified synthetic oligosaccharides were verified by ¹H-nuclear magnetic resonance spectroscopy; 500–2000 nmol of each of the oligosaccharides was converted to the corresponding *N*-hydroxysuccinimide ester by use of *N,N,N',N'*-tetramethyl-(succinimido)uronium tetrafluoroborate and subsequently was linked to the lysine residues of bovine serum albumin (BSA), as described elsewhere [4]. The average loading of oligosaccharides per mol of BSA was 14 mol LewisX, 12 mol LDN, 3 mol LDN-F, and 11 mol LDN-DF, as determined by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry.

Lipopolysaccharide (LPS) contamination. The LPS content of the glycolipid preparations and neoglycoconjugates was determined using a limulus amoebocyte lysate assay (LAL COATEST Endotoxin; Chromogenix). No endotoxin could be detected in the glycolipid preparations. The endotoxin concentration in all neoglycoconjugate preparations was <1 ng/mL. To avoid the effects of endotoxin on cytokine production, all cell-culture experiments were performed in the presence of 10 μ g/mL polymyxin B sulfate (Sigma). The presence of this LPS inhibitor was sufficient to completely abrogate the cytokine response of the PBMC to 5 ng/mL LPS.

Stimulation of PBMC. PBMC were isolated and cryopreserved as described elsewhere [26]. Cryopreserved cells were cultured in 96-well flat bottom plates (Nunc) at 10⁶ cells/well in 200 μ L RPMI 1640 culture medium, as detailed elsewhere [26]. The cells were stimulated with 5 nmol/mL free carbohydrates, 5 μ g/mL BSA-conjugated carbohydrates, or 10 μ g/mL glycolipids. Glycolipids were dissolved in 0.1% dimethyl sulfoxide by water bath sonication. Cells were incubated at 37°C in 5% CO₂, and supernatants were collected 20 h after stimulation. Cytokine levels were determined in the super-

Oligosaccharide	Sequence	MAb
LewisX	Gal β 1-4GlcNAc-R Fuc α 1-3	291 4D10
LDN	GalNAc β 1-4GlcNAc-R	114 2H12
LDN-F	GalNAc β 1-4GlcNAc-R Fuc α 1-3	294 2A1
LDN-DF	GalNAc β 1-4GlcNAc-R Fuc α 1-3 Fuc α 1-2	114 5B1

Figure 1. Structures of oligosaccharides and their interaction with specific monoclonal antibodies (MAbs). LDN, LacdiNAc (GalNAc β 1-4GlcNAc-R); LDN-DF, difucosylated LDN; LDN-F, fucosylated LDN; R, spacer (carboxymethyl octyl group) used to couple the carbohydrates to bovine serum albumin.

natants by ELISA, using commercial kits (CLB) according to the manufacturer's recommendations.

Identification of cytokine-producing cells. PBMC were stimulated for 6 h with schistosome egg glycolipids, and IL-10-secreting cells subsequently were stained using an IL-10 secretion assay (kindly provided by Dr. Mario Assenmacher, Miltenyi Biotec, Bergisch Gladbach, Germany). As a positive control, cells were stimulated with LPS (Sigma), *Staphylococcus enterotoxin B* (SEB; Sigma), and *Staphylococcus aureus* crude cell suspension (SAC; Calbiochem). To reduce nonspecific binding of fluorochrome-conjugated antibodies, the cells were preincubated with a mixture of blocking Fc γ receptor antibodies (CD16 [Fc γ III], CLB; CD32 [Fc γ II], Medarex; and CD64 [Fc γ I], Diaclone). Cells then were stained with CD14-fluorescein isothiocyanate (FITC; BD Biosciences), CD3-peridinin chlorophyll protein (PerCP; BD Biosciences), CD19-allophycocyanin (BD Biosciences), and IL-10-phycoerythrin (PE; Miltenyi Biotec). The cell preparation was enriched for IL-10-producing cells by magnetic cell sorting (MACS) according to the protocol of the IL-10 secretion assay, using only 1 magnetic separation step. Staining of cell-surface markers was analyzed using a FACSCalibur (Becton Dickinson). To exclude dead cells from the fluorescence-activated cell sorter (FACS) data analysis, a life gate was set, based on forward scatter (FSC)-side scatter (SSC) plots, and a surface-marker-negative gate was used. For monitoring IL-10-producing monocytes, CD3⁺ and CD19⁺ cells were excluded; in the case of T cells, CD19⁺ and CD14⁺ cells were excluded, and, for B cells, CD3⁺ and CD14⁺ cells were excluded. For calculations of percentages of IL-10-producing target cells, FACS analysis also was performed before enrichment of IL-10-producing cells.

IL-6 and tumor necrosis factor (TNF)- α were stained intracellularly after stimulation for 6 h in the presence of 10 μ g/mL brefeldin A, using anti-TNF- α -PE (BD Biosciences) and anti-IL-6-PE (BD Biosciences) as cytokine detection antibodies, as described by Yamamura et al. [27]. CD14-FITC, CD3-PerCP, and CD19-FITC (Dako) were used as surface-marker antibodies. To exclude dead cells from the analysis, a life gate and a surface-marker negative gate were used in the analysis of monocytes and T cells; a lymphocyte gate based on FSC-SSC was used for the analysis of B cells.

MAbs recognizing defined carbohydrate structures. MAbs were obtained from fusions of spleen cells of mice infected with *S. mansoni* (in the case of MAbs 114 2H12C [IgM], 114 5B1 [IgG1], 147 2G9 [IgG1], and 54 5C10 [IgG3]) [28, 29] or from fusions of spleen cells of mice immunized with hatching fluid of *S. mansoni* eggs (in the case of MAbs 294 2A1 [IgM] and 291 4D10 [IgM]) [30]. Binding specificity of the MAbs was monitored using the neoglycoconjugates described above. MAbs 114 2H12C, 291 4D10, and 114 5B1 specifically recognized the LDN conjugate, the LewisX conjugate, and the LDN-DF conjugate, respectively, whereas 294 2A1 was found to bind to both the LDN-F and LDN-DF conjugates [4]. The MAb recognition pattern of structures present in schistosome life-cycle stages was monitored by immunofluorescence assay, using frozen liver sections of *S. mansoni*-infected hamsters and of adult worms fixed with Rossman's fixative. An overview of the recognition pattern of the MAbs used in this study has been published elsewhere [4].

Analysis of MAb binding on schistosome glycolipids. PolySorp microtiter plates (Nunc) were coated overnight at room temperature with methanol-dissolved glycolipids (33 ng/well for worm glycolipids and 17 ng/well for egg glycolipids). Lipid-coated plates were air-dried overnight. Plates were blocked by a 1-h incubation with 200 μ L blocking solution (0.07% [w/v] bovine nonfat dry milk in PBS). After blocking, plates were washed 5 times with PBS and 0.01% Tween-20. Hybridoma cell supernatants containing the MAbs mentioned in figure 1 were applied undiluted at 50 μ L/well. After a 1-h incubation, plates were washed as described above and were incubated for 1 h with horseradish peroxidase (HRP)-conjugated rat anti-mouse antibodies. All incubations listed above were done at 37°C while shaking. After washing, assays were developed at room temperature with 3,3',5,5' tetramethylbenzidine as substrate. Reactions were stopped by adding 2 M H₂SO₄, and absorbance was read at 450 nm in an automated plate reader.

Results

Schistosome egg glycolipids induce cytokine production in human PBMC. To determine whether glycolipids extracted from *S. mansoni* can interact with the cells of the immune system,

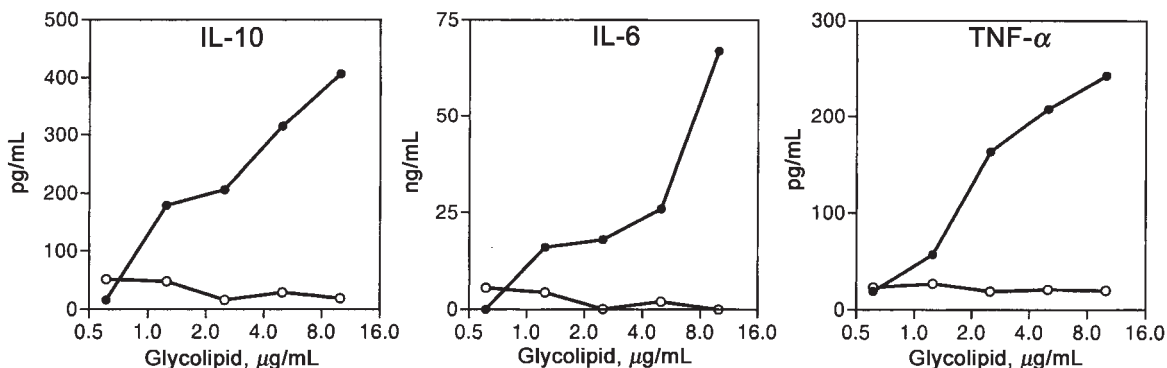


Figure 2. Cytokine production by peripheral blood mononuclear cells (PBMC) of 10 healthy donors stimulated with various concentrations of glycolipid-containing extracts from *Schistosoma mansoni* eggs (●) or adult worms (○). Cytokine production was measured in supernatants by ELISA after 20 h of stimulation. All donors showed comparable response patterns; a representative result is shown. IL, interleukin; TNF, tumor necrosis factor.

we stimulated PBMC of nonexposed individuals with different concentrations of glycolipids derived from eggs or adult worms and measured cytokine production. The PBMC produced IL-10, IL-6, and TNF- α after stimulation with the egg glycolipid preparation but not in response to worm glycolipids, which indicates that egg glycolipids are capable of stimulating innate immune responses. Cytokine production after stimulation with egg glycolipids was dose dependent (figure 2). The compound inducing cytokine production was heat stable, which is in agreement with the notion that the active compound is a lipid and not a protein (data not shown). Using mass spectrometry, we found trace amounts of phosphatidyl choline and phosphatidyl ethanolamine in the egg glycolipid fraction; however, other fractions containing these phospholipids in larger amounts did not have any cytokine-inducing activity, which demonstrates that cytokine production measured in response to the egg glycolipid fraction was not in-

duced by phospholipids and suggests that the cytokine-inducing activity can indeed be attributed to the glycolipids present in this fraction (data not shown).

IL-10 induced by schistosome egg glycolipids is produced by monocytes. To study which cell type was responsible for the production of the cytokines measured in response to schistosome egg glycolipids, we stimulated PBMC with egg glycolipids for 6 h and subsequently stained for IL-10-producing cells by use of an IL-10 secretion assay. As a positive control, PBMC were stimulated with a combination of high concentrations of LPS, SAC, and SEB (LPS/SEB/SAC). The cell preparations were enriched for IL-10-secreting cells by MACS, and IL-10 secretion and expression of CD3, CD19, and CD14 were analyzed by FACS. The percentages of B cells (CD19⁺), T cells (CD3⁺), and monocytes (CD14⁺) secreting IL-10 were assessed using both enriched and nonenriched cell populations (figure 3A). The results show

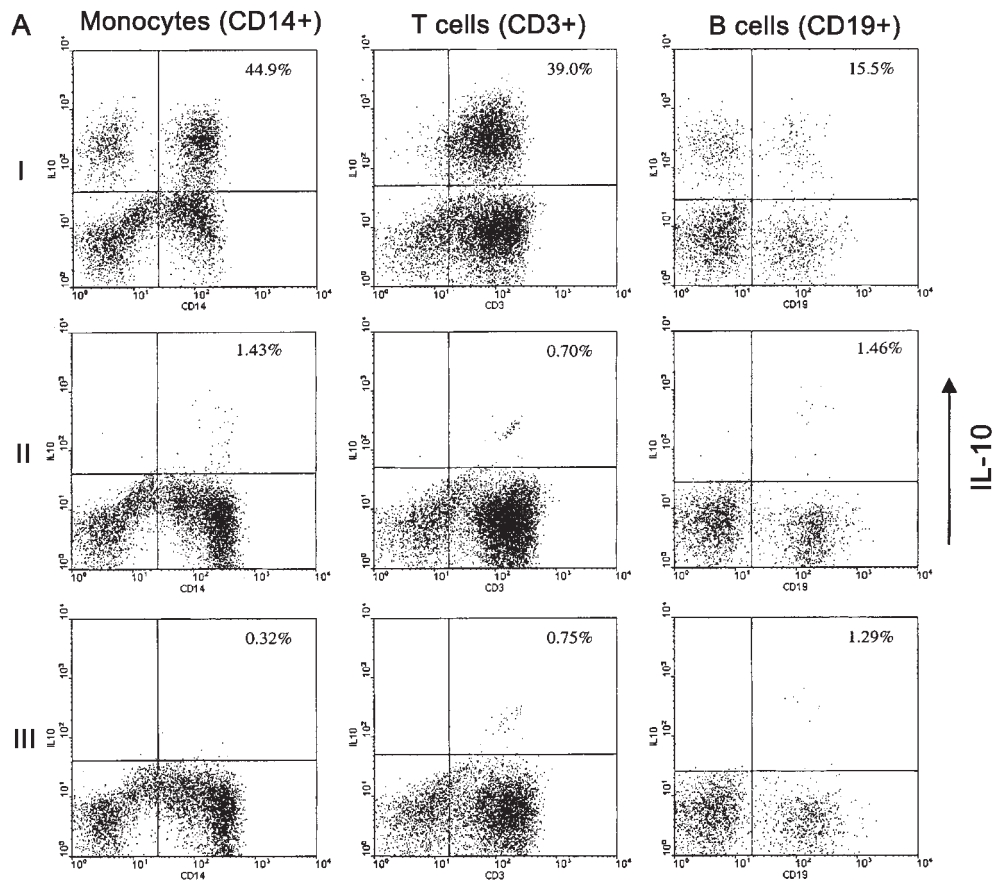


Figure 3. Cytokine production by peripheral blood mononuclear cells (PBMC) of a healthy donor. PBMC were stimulated with 100 ng/mL lipopolysaccharide (LPS), 100 ng/mL *Staphylococcus aureus* crude cell suspension (SAC), and 100 ng/mL *Staphylococcus enterotoxin B* (SEB) (row I); 10 μ g/mL schistosome egg glycolipids in the presence of 10 μ g/mL polymyxin B (row II); or polymyxin B containing medium alone (row III) for 6 h. Cytokine production was analyzed subsequently using an interleukin (IL)-10 secretion assay (A) or intracellular staining of IL-6 (B) and tumor necrosis factor (TNF)- α (C), as described in Materials and Methods. The phenotype of cytokine-producing cells was monitored using flow cytometry. Percentages of cytokine-producing cells are indicated. For IL-10 detection, the cell preparations were enriched for IL-10-producing cells, and percentages of IL-10-secreting cells in enriched samples are indicated in the figure. Percentages of IL-10-producing monocytes in nonenriched samples were 8.89%, 0.29%, and 0.07% for LPS/SAC/SEB, schistosome egg glycolipids, and unstimulated controls, respectively. Results for 1 donor are shown; similar results were obtained using PBMC from 2 other donors.

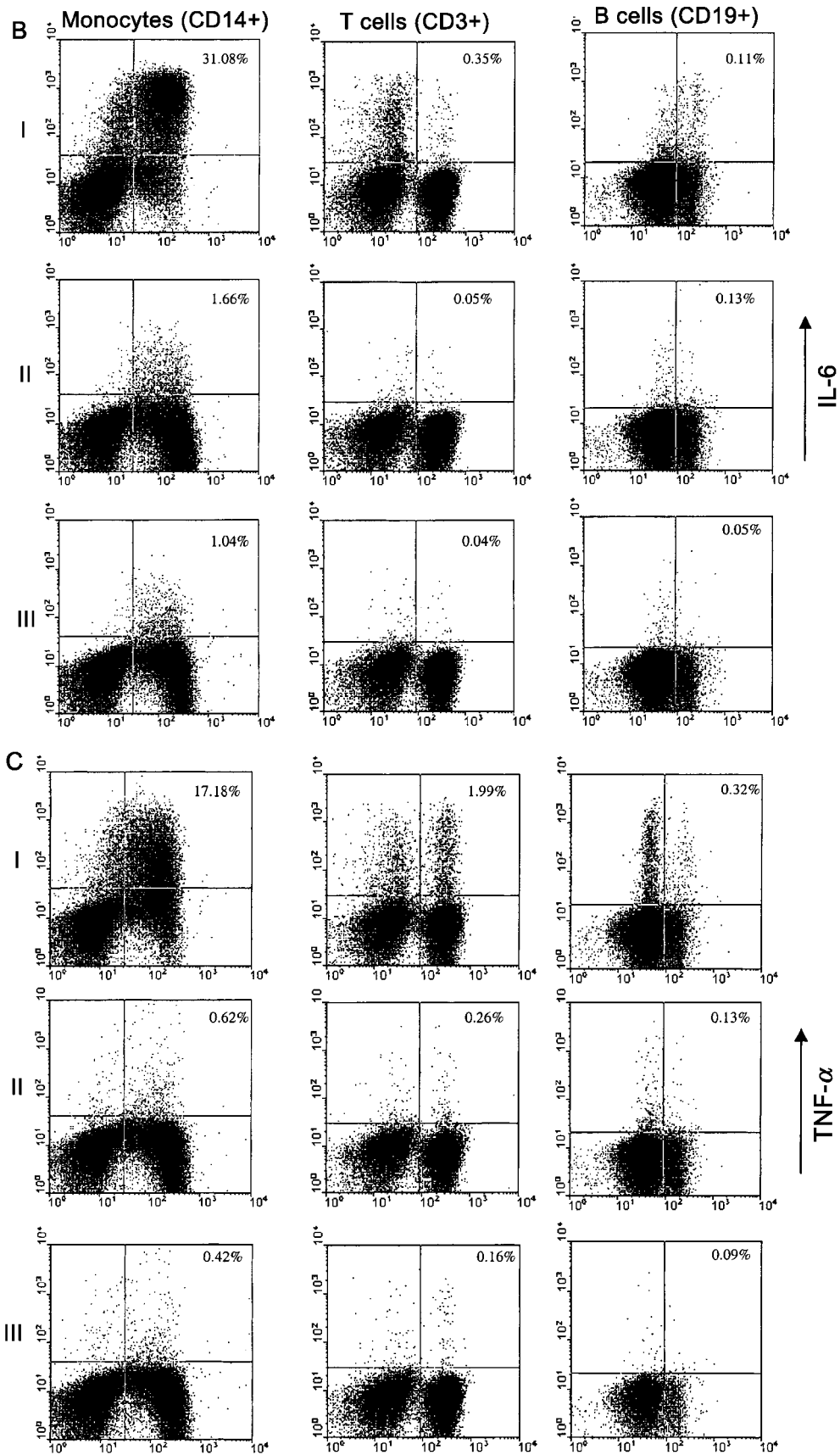


Figure 3. (Continued.)

that, in response to LPS/SEB/SAC, IL-10 production was seen in T cells, B cells, and monocytes, whereas, in response to schistosome egg glycolipids, IL-10 secretion was significantly elevated in monocytes only ($P = .0155$), compared with the unstimulated control population. For T and B cells, the percentages of IL-10-producing cells were comparable in the egg glycolipid-stimulated and -unstimulated populations ($P = .6525$ and $P = .3692$, respectively), indicating that, within PBMC, IL-10 production in response to schistosome egg glycolipids is induced solely in monocytes. To determine whether IL-6 and TNF- α also were produced by monocytes, production was monitored by intracellular staining (figure 3B and 3C). Polyclonal stimuli (LPS and SEB) led to production of IL-6 by monocytes only (figure 3B), whereas TNF- α was detected in monocytes, as well as in T cells, albeit to a lesser extent (figure 3C). IL-6 production in response to egg glycolipids was significantly elevated in monocytes only ($P = .026$), not in T and B cells ($P = .423$ for T cells; $P = .094$ for B cells; figure 3B), whereas TNF- α production in response to egg glycolipids could not be attributed conclusively to a particular cell type (figure 3C).

Cytokines also were measured in cell culture supernatants (data not shown), and, when the results were expressed as the ratio of cytokines produced in response to polyclonal stimuli (i.e., cytokines produced in response to egg glycolipids), the following results were obtained: for IL-10, 40; for IL-6, 10; and for TNF- α , 10. These results agree well with data from FACS analysis showing staining for secretion of IL-10 or intracellular IL-6 and TNF- α (figure 3).

LDN-DF is a major epitope in schistosome egg glycolipids but not worm glycolipids. To characterize the carbohydrate moieties present in glycolipids of schistosome eggs and adult worms, the binding of MAbs with known specificities [4] (figure 1) generated in our laboratory was measured. The binding patterns of the MAbs to the glycolipids are depicted in figure 4. These results show that schistosome egg glycolipids and worm glycolipids bear LewisX, LDN, and LDN-F epitopes. However, of the structures tested, LDN-DF was the most abundant carbohydrate epitope in egg glycolipids but was poorly represented in worm glycolipids.

LDN-DF induces cytokine production in human PBMC. To test whether the carbohydrate structures present on glycolipids (figure 1) could interact with the immune system to induce cytokine production, we measured the response of PBMC to enzymatically synthesized carbohydrates. When PBMC were stimulated with the synthesized oligosaccharides, no cytokine responses could be measured. However, since naturally occurring glycolipids are present in membrane structures and, therefore, effectively are multivalent, we considered that cross-linking may be required for the induction of cytokine responses. We then tested the same carbohydrates after coupling them to the lysine residues of BSA [4]. When PBMC from the same donors were stimulated with the neoglycoconjugates IL-10, IL-6, and TNF- α , production was stimulated (figure 5), indicating that cross-linking

is indeed required for the induction of cytokine production. Although all the carbohydrate-BSA conjugates stimulated some level of IL-10, IL-6, and TNF- α production, it was clear that LDN-DF was the most potent stimulus in each donor. Therefore, the differential capacity of the egg and adult worm glycolipids to stimulate cytokine responses may be explained by the prominent presence of LDN-DF in egg glycolipids, compared with worm glycolipids.

Discussion

Our current data show that schistosome glycolipids and carbohydrates present in the glycolipids can induce pro- and anti-inflammatory cytokine responses in nonexposed individuals, indicating a role for these structures in stimulating innate immunity. A number of distinct carbohydrate moieties, such as LewisX, LDN, LDN-F, and LDN-DF, were shown to be present on egg glycolipids. By using neoglycoconjugates prepared by enzyme-assisted synthesis, it was possible to test defined carbohydrate structures and to show, for the first time, that LDN-DF, in particular, has the capacity to stimulate monocytes to release cytokines. Vellupillai et al. [14] reported that PBMC of schistosome-infected individuals produce IL-10 after stimulation with lacto-*N*-fucopentaose III (LNFPIII) containing the LewisX trisaccharide conjugated to BSA. However, no responses to LNFPIII could be measured in uninfected individuals. This is in agreement with our data, which show that nonexposed subjects respond poorly to LewisX, compared with LDN-DF. This

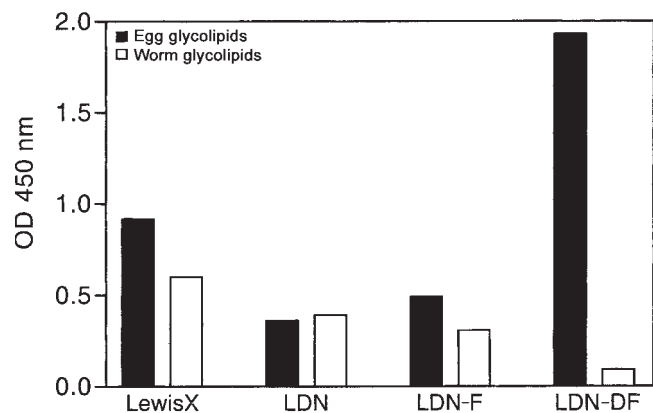


Figure 4. Binding of monoclonal antibodies (MAbs) to specific carbohydrate structures present in glycolipid preparations of *Schistosoma mansoni* eggs and adult worms, monitored by ELISA. Binding of MAbs 114 2H12C, 291 4D10, and 294 2A1 to the glycolipid preparations was measured to determine the presence of the carbohydrate epitopes LacdiNAc (LDN [GalNAc β 1-4GlcNAc-R]), LewisX, fucosylated LDN (LDN-F), and difucosylated LDN (LDN-DF), respectively. Using 2 antibodies that did not recognize any of the neoglycoconjugates (147 2G9 and 54 5C10, recognizing carbohydrate epitopes of the schistosome antigens circulating anodic antigen and circulating cathodic antigen, respectively), we measured optical density (OD) values of 0.058 and 0.061, respectively.

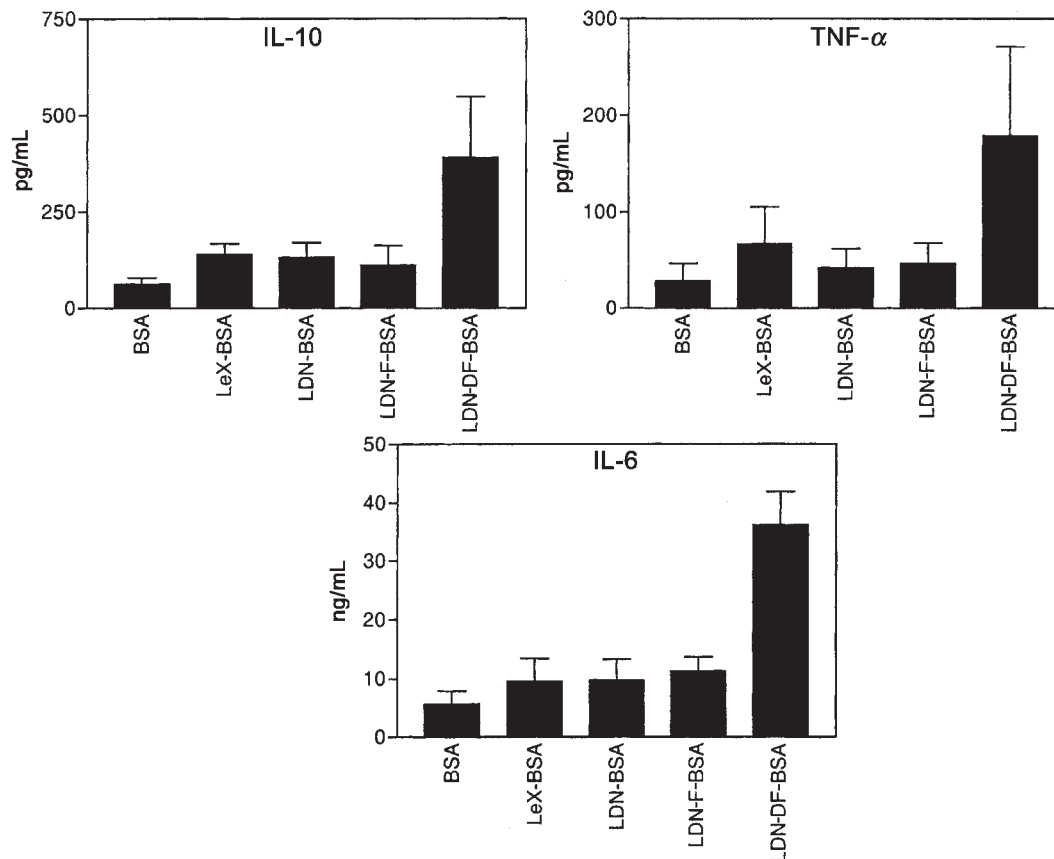


Figure 5. Cytokine responses of peripheral blood mononuclear cells (PBMC) of healthy donors stimulated with enzymatically synthesized carbohydrates that were coupled to bovine serum albumin (BSA). PBMC were stimulated with 5 $\mu\text{g}/\text{mL}$ BSA-coupled carbohydrates, and cytokine production was measured in supernatants after 20 h of stimulation. Graphs show mean values + SE, calculated from responses of 4 donors. IL, interleukin; LDN, LactidNAc (GalNAc β 1-4GlcNAc); LDN-DF, difucosylated LDN; LDN-F, fucosylated LDN; LeX, LewisX antigen; TNF, tumor necrosis factor.

may suggest that IL-10 production in response to LewisX is part of the adaptive immune response mounted during schistosome infection, whereas responses to LDN-DF represent interaction with the innate immune system.

The ability of glycolipids to interact with the human immune system has been reported in several studies [31–33]. T cell proliferation can be suppressed by a range of gangliosides, including GD3 (NeuAc α 2-8NeuAc α 2-3Gal β 1-4Glc β 1-Cer) in particular, whereas GD3, GD1a, GM3, GM2, and GM1 all are effective in reducing TNF production in PBMC; the effect is dependent on the presence of both the lipid and the sugar moiety [32]. With respect to parasite glycolipids, it has been reported elsewhere [33] that phosphocholine (PC)–containing zwitterionic glycosphingolipids derived from the porcine parasitic nematode *Ascaris suum* can induce IL-1, TNF- α , and IL-6 production. However, the removal of the PC group abolished the biological activity of these structures, granting the reactivity to the PC group, rather than to the carbohydrate structures [33]. Taken together, it is clear that a spectrum of immunomodulatory activities can be at-

tributed to glycolipids, and our work highlights the ability of oligosaccharides, a difucosylated epitope in particular, to stimulate monocytes to produce a mixture of pro- and anti-inflammatory cytokines. The importance of a fucose moiety has been highlighted in a study by Okano et al. [15] showing that not only the presence of fucose on a Gal β 1-4GlcNAc structure but also the type of linkage influences the immunological activity of Lewis-family sugars. The results of the present study, which used LDN-based structures, not only underlines the importance of fucose but also shows that the presence of a unique difucosylated epitope enhances reactivity above the equivalent monofucosylated structure.

Pro- and anti-inflammatory cytokines have been shown to have major effects on granuloma formation and on disease progression in schistosomiasis. TNF- α seems to be associated with pathogenesis of schistosomiasis [10, 34], whereas IL-6 and IL-10, which are released when schistosome eggs are encountered by the immune system, may moderate the extent of disease progression [35–37]. In the present study, we show that glycolipid

components of eggs at a concentration of 1.25 $\mu\text{g}/\text{mL}$, an amount originating from <100 eggs, can stimulate cytokine production by 10^6 PBMC. Considering that the adult female schistosome worm produces, on average, 300 eggs per day, it is very likely that the glycoconjugates will indeed play an important role in directing immune reactions. Thus, the interaction between the immune system and schistosome eggs, superimposed on host genetics, may form an important decision point with respect to the outcome of immunopathologic processes. The notion that LDN-DF may interact with innate immunity is supported by the presence of this structure both on the *S. mansoni* shell and in egg excretion and secretion [4], which is accessible to interaction with the immune system.

Recently, considerable attention has been given to the recognition of certain molecular patterns present in invading pathogens, which can lead to a rapid onset of polarized immune responses after infection. For bacteria, Toll-like receptors (TLRs) have been shown to play an important role in such recognition and often recognize nonprotein molecules [1, 3, 38]. Now that carbohydrate structures that are capable of stimulating blood monocytes have been defined, it will be possible to study their interaction with not only TLRs but also with other possible specific receptors. It is of interest that the free synthetic oligosaccharides were unable to stimulate monocytes to produce cytokines, indicating that multivalency is essential.

Within a population exposed to schistosome infections, the heterogeneity of reactions to stimuli considered in the present study may be one of the factors underlying the spectrum of clinical manifestations observed. The further fractionation and characterization of glycoconjugates in schistosomes, as well as their specific interaction with defined host receptors, may provide novel insights into mechanisms that mediate egg-induced immunopathologic processes.

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